UNIVERSITI PUTRA MALAYSIA

INDOLE-3 ACETIC ACID PRODUCING RHIZOBACTERIA AND ITS POTENTIAL TO ENHANCE GROWTH OF SWEETPOTATO (IPOMOEA BATATAS L.)

LUSI MAIRA

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By

LUSI MAIRA

Thesis Submitted in Fulfilment of the Requirement for the Degree of Master of Agricultural Science in the Faculty of Agriculture Universiti Putra Malaysia

December 2000

DEDICATION

This work is dedicated

to

my Late Father, Nazir St. Mudo

whose commitment to scientific studies was my inspiration

to

my mother Reno Gadis

and to

my husband Indra Jaya Malik, our daughter Rezkya

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Agricultural Science

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Chairman : Dr. Radziah Othman

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The problem in sweetpotato cultivation is yield reduction with continuous planting, which can be attributed to several factors such as soil degradation, pathogenic effects, and allelopathic effects. One of the approaches to alleviate this problem is by inoculating plants with indole-3-acetic acid (IAA) producing rhizobacteria to enhance growth of sweetpotato.

The study consisted of four experiments. Experiment I was the isolation of rhizobacterial strains from sweetpotato rhizosphere and their performance in sweetpotato root exudate culture. Experiment II was the selection of IAA producing rhizobacteria and their effects on sweetpotato growth. Experiment III was the determination of the effect of pH and carbon sources on growth of rhizobacteria isolated from sweetpotato rhizosphere. Experiment IV was the determination of the effects of L-tryptophan (TRP) on performance of IAA producing rhizobacteria on growth of sweetpotato.

From the isolation procedures, 115 rhizobacterial isolates were collected from different varieties of sweetpotato grown on different soils. They were grouped into 6 types according to colour of the colonies which consist of purple, milky white, translucent, orange, yellow and brown. The study showed that 48 isolates (41.74 %) were able to grow on medium containing sweetpotato root exudate of which, 18 isolates (37.5%) were able to produce IAA in culture medium which ranged between 4 to 8 μg/mL.

The first pot experiment showed that 6 isolates (SPR 66, SPR 83, SPR 85, SPR 88, SPR 100 and 90-166) were able to stimulate the initial growth of sweetpotato vines. Three of the isolates namely SPR 66, SPR 83 and SPR 100 showed positive responses on media with wide range of pH and different carbon sources. The optimum pH of medium for rhizobacterial growth was 6.75, and the most favourable carbon source was malic acid.

The second pot experiment showed that isolate SPR 66 increased the shoot dry weight, chlorophyll content and total leaf area of shoots, while isolate SPR 100 increased the dry weight and volume of roots, the storage root formation, nutrient concentration in shoots and soil nutrient contents. The addition of L-TRP to all selected rhizobacterial isolates enhanced growth and yield of sweetpotato. It related to the improved of soil chemical properties and microbial activity.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains Pertanian.

BAKTERIA RIZOSFERA PENGHASIL INDOLE-3 ACETIC ACID DAN KEUPAYAANNYA DALAM MENINGKATKAN PERTUMBUHAN KELEDEK (*IPOMOEA BATATAS* L)

Oleh

LUSI MAIRA

Disember 2000

Pengerusi : Dr. Radziah Othman

Fakulti : Pertanian

Masalah bagi penanaman pokok keledek ialah berlakunya pengurangan hasil apabila ditanam secara berterusan, yang berpunca daripada beberapa faktor seperti degradasi tanah, kesan patogen dan kesan allelopati. Salah satu pendekatan yang digunakan untuk meningkatkan tumbesaran pokok keledek ialah dengan menginokulasikan bakteria rizosfera penghasil indole-3-acetic acid (IAA).

Empat kajian telah dijalankan untuk melihat kesan bakteria ini. Kajian I ialah pengasingan rhizobakteria daripada rizosfera keledek dan keupayaannya untuk tumbuh pada media yang mengandungi eksudat akar keledek. Kajian ke II ialah pemilihan rhizobakteria yang menghasilkan IAA dan mengkaji kesan rizobakteria pada pokok keledek. Kajian ke III ialah kesan pH dan sumber karbon pada pertumbuhan rizobakteria dan kajian ke IV ialah kesan L-tryptophan (TRP) pada rhizobakteria untuk tumbesaran pokok keledek.

Daripada prosedur pemencilan sejumlah 115 rhizobakteria telah diasingkan daripada keledek pelbagai varieti yang ditanam pada jenis dan lokasi tanah yang berlainan. Bakteria berkenaan dikelaskan kepada 6 jenis berdasarkan warna koloni yang terdiri dari warna ungu, putih, lut cahaya, jingga, kuning dan coklat. Kajian menunjukkan 48 isolat (41.74 %) mampu tumbuh pada media yang mengandungi eksudat keledek, 18 isolat (37.5 %) berkebolehan menghasilkan IAA di dalam media kultur dengan julat antara 4-8 μg/mL.

Kajian berpasu yang pertama menunjukan 6 isolat (SPR 66, SPR 83, SPR 85, SPR 88, SPR 100 and 90-166) dapat merangsang tumbesaran pokok keledek. Tiga daripada isolat ini SPR 66, SPR 83, dan SPR 100 menunjukan tindak balas yang positif terhadap media dengan pelbagai pH dan sumber karbon. pH media optimum yang paling sesuai untuk tumbesaran rhizobakteria ialah 6.75, manakala asid malik merupakan sumber karbon yang paling digemari.

Pada kajian berpasu kedua diperolehi isolat SPR 66 berkebolehan meningkatkan berat kering bahagian atas, kandungan klorofil dan jumlah keluasan daun, manakala isolat SPR 100 memberi kesan kepada berat kering dan isipadu akar, pembentukan ubi, kepekatan nutrien pada bahagian atas dan kandungan nutrien tanah. Manakala pemberian L-TRP kepada semua isolat terpilih berkesan meningkatkan tumbesaran dan hasil pada pokok keledek. Peningkatan tumbesaran yang berlaku berkaitan dengan peningkatan keadaan kimia tanah dan aktiviti mikroorganisma dalam tanah.

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Finally, I wish to express my deepest appreciation to my beloved husband Indra Jaya, daughter Rezkya Amanda, mother, sisters, brothers, and the rest of the family members, for their patience and endurance and for standing by me through those difficult times. To them, I dedicate this work.

I praise the Al-mighty Allah, for enabling me to accomplish the task that was assigned to me.

I certify that an Examination Committee met on 4th December 2000 to conduct the final examination of Lusi Maira on her Master of Agricultural Science thesis entitled "Indole-3 Acetic Acid Producing Rhizobacteria and Its Potential to Enhance Growth of Sweetpotato (*Ipomoea batatas* L)" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The committee recommends that the candidate be awarded the relevant degree. Members of Examination committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Master of Agricultural Science.

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I hereby declare that the thesis is based on my original work except for quotations and citations which, have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

LUSI MAIRA

Date: 14-02-2001

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CHAPTER 1

INTRODUCTION

Sweetpotato *(Ipomoea batatas* L.) is an important food crop grown mainly for its starch storage root and a wide range of protein contents that ranged from 0.5 to 9.1 % dry weight (Yeoh and Truong, 1996). The starchy, tuberous roots are the major source of food and the leaves and also useful source of green vegetable in some countries. The boiled sweetpotato roots has similar energy as boiled rice and beans, high vitamins and minerals (Paul and Southgate,1979; Woolfe, 1992), and can be used as chicken feed (Agwunobi, 1995; Ravindran and Sivakanesan, 1996).

In the normal practice, sweetpotato is being cultivated in a mono-cultured system. However, it has been observed that continuous cropping for many years on the same area has caused reduction in yield. In Perak Malaysia, which is the main area of sweetpotato cultivation, it was observed that yield of sweetpotato decreased after four seasons of cropping (Saad, Personal comm., 1997). Similar observation was recorded in several other countries. Yield of sweetpotato in Louisiana State, USA was observed to reduce when grown continuously for more than four years (Miller *et al.*, 1961). Growers in Texas reported excessive sweetpotato root cracking which led to low yield in fields that has been successively planted with sweetpotato (Peterson and Speights, 1964).

These observations indicated that the problems of sweetpotato yield reduction were not only related to soil fertility. It has been suggested that it could also be due to increased pathogenic activity. However, such problems of pest and diseases could be controlled in a well-managed farm indicating that there could be other problems. Production of allelo-compounds by plants and its residue in continuous cropping could be another important factor inhibiting plant growth and yield (Einhellig ,1985).

Many methods have been suggested to overcome the yield reduction, such as addition of fertilizer, crop rotation, mixed or inter-cropping and addition of organic matter. One of the methods used to increase plant growth is plant inoculation with beneficial microorganisms. Several species of bacteria, fungi and actinomycetes have been known to be beneficial to plant growth. The most studied microorganisms are the plant growth-promoting rhizobacteria (PGPR), a group of rhizosphere bacteria that aggressively colonize the roots (Schroth and Hancock, 1982; Kennedy, 1998).

Microorganisms are considered the primary sources of biologically active substances in soil. They are able to synthesize a diverse group of substances that stimulate plant growth by direct or indirect modes of action(Frankenberger and Arshad, 1995). The metabolites produced by soil microorganisms may influence the growth and development of plants through plant growth regulators, vitamins and inhibitors (Strzelczyk and Burdziej, 1984; O'Gara *et al.*, 1994 and Astrom *et al.*, 1993). The plant hormones

(phytohormones) are produced endogenously by plants and influence physiological processes of plants at very low concentrations. One of the phytohormones that can be synthesized by microorganisms is indole-3 acetic acid (IAA).

Some of the well known PGPR are *Azotobacter, Azospirillium, Pseudomonas* and *Serratia* (Kloepper, 1993 and Okon, 1985). Although PGPR has been shown to benefit various plants, there is limited information on the presence and activity of other PGPR on sweetpotato. Yassin *et al.* (1994) have found that application of *Azospirillum* on sweetpotato resulted in positive plant responses and higher yield. *Azospirillum* and other bacteria were found to be a potential bio-fertilizer for sweetpotato as it increased yield through the N_2 fixing process and other beneficial mechanisms (Saad *et al.*, 1996).

This study hypothesized that rhizobacteria from sweetpotato rhizosphere could influence the growth and yield of sweetpotato by producing the phytohormone indole-3 acetic acid. Laboratory and glasshouse experiments were conducted with the following objectives:

i) To isolate IAA producing rhizobacterial strains from sweetpotato rhizosphere.

ii) To determine the effects of pH and carbon sources on growth of the bacterial isolates.

iii) To determine the effects of IAA producing rhizobacteria on growth of sweetpotato.

iv) To determine the effects of L-tryptophan on the performance of IAA producing rhizobacteria on growth of sweetpotato and soil chemical properties.

CHAPTER 2

LITERATURE REVIEW

Sweetpotato Production

Sweetpotato in General

Sweetpotato is a tropical and subtropical crop originated from Central America or North-western South America. It also grows in the warmer areas of the temperate regions. It is a warm weather crop, grows best at temperatures above 24 ^{0}C and is seriously retarded when temperature fall below 10 ^{0}C (Bouwkamp, 1985). Currently, sweetpotato is being produced in large scale, contributing 85 % of the world tuber yield (CIP, 1994). Although sweetpotato is a perennial plant, it is normally grown as an annual. The plant is usually propagated from vine cutting and its growth and yield are influenced by several factors.

Sweetpotato may be successfully produced in a wide range of soil type. However, the storage root shape and appearance are best when sweetpotato is grown in light, sandy or sandy loam, and does poorly on clay soils (Bouwkamp, 1985). Good drainage systems include irrigation are essential since the crop cannot withstand water logging and drought. Under water stress condition the leaf number, leaf area and stem length have been